Season of surgery, breast cancer survival and vitamin D: an ecological, open cohort study

Journal:	*BMJ Open*
Manuscript ID:	bmjopen-2011-000358
Article Type:	Research
Date Submitted by the Author:	30-Sep-2011
Complete List of Authors:	Teilum, Dorthe; Brystkirurgisk Klinik PBB Bjerre, Karsten; Danish Breast Cancer Cooperative Group, Department 2501, Tjønneland, Anne; Institute of Cancer Epidemiology,, Dannish Cancer Society Kroman, Niels; Rigshospitalet,
Primary Subject Heading:	Surgery
Secondary Subject Heading:	Oncology
Keywords:	Breast cancer, Breast surgery < SURGERY, Vitamin D

Season of surgery, breast cancer survival and vitamin D: an ecological, open cohort study

Dorthe Teilum, Karsten D Bjerre, Anne M Tjønneland and Niels Kroman

Abstract

Background: Vitamin D has been suggested to influence the incidence and prognosis of breast cancer, and studies have found better overall survival after diagnosis for breast cancer in summer-autumn, where the vitamin D level are expected to be highest.

Objective: To compare the prognostic outcome for early breast cancer patients operated at different seasons of the year.

Design: Open population-based cohort study.

Setting: Danish women operated 1978-2010.

Cases: 80,149 adjusted for age at surgery, tumor size, axillary lymph node status, and hormone receptor status.

Statistical analysis: The association between overall survival and season of surgery was analyzed by Cox proportional hazards regression models, at survival periods 0-1, 0-2, 0-5 and 0-10 years after surgery. A two sided p-value < 0.05 was considered statistical significant.

Results: In the adjusted model in the periods 0-1 year after operation the overall survival ratio was 0.96 (P< 0,05) for patients operated in the autumn. Two, five and ten years after surgery the difference disappeared (P>0.05).

Limitations: Season is a surrogate measure of vitamin D.

Conclusion: We found no evidence of a seasonal variation in the survival after surgery for early breast cancer. Lack of seasonal variation in this study does not necessarily mean, that vitamin D is of no importance for the outcome for breast cancer patients.

Article summary

Article focus:

- Breast cancer survival and season of surgery

Key message:

- No evidence of a seasonal variation

Strengths of this study:

- The sample size (>80.000 cases)
- The population-based approach in a limited geographic area
- The prospectively collected characteristics of tumor and lymph node status
- The long follow-up (mean 9.3 years)

Limitations of this study:

- The lack of information about vitamin D in the individual patient at the time of surgery.
- It is not known, whether vitamin D levels of the breast cancer patients follow that of the background population.

Introduction

Over the past decades, ecological studies have inspired to the hypothesis, that exposure to sunlight and hence difference in blood levels of vitamin D, may influence both risk and prognosis for breast cancer [1;2]. The hypothesis has been supported by several in vitro and animal studies [3;4], in addition to case-control and cohort studies with measurements of blood levels of 25 hydroxy-vitamin D (25(OH)D) [5-13] although not all studies including two meta-analyses could support these findings,[14-17]. The prognosis of breast cancer has been found to vary with the season for diagnosis [18-21]. Three studies found, that patients diagnosed in summer-autumn had a better disease outcome than patients diagnosed in winter-spring [22-24] and one study found a higher overall mortality for patients diagnosed in August compared to those diagnosed in January [25].

In Denmark, positioned at 55-58^0 northern latitude, there is not sufficient sun to produce vitamin D in the human skin during 6-8 months of the year. Measurements of vitamin D in healthy, Danish volunteers demonstrate a pronounced seasonal variation of vitamin D with a maximum in August and a minimum in March-April, which indicates, that the content of vitamin D in the average Danish diet could not compensate for the lack of sun induced vitamin D production during wintertime [26].

If the amount of vitamin D in the blood at the time of the operation is important for the overall survival (OS), it should be both easy and inexpensive to adjust preoperatively. The aim of this study is to compare the prognostic outcome for early breast cancer patients, diagnosed and operated at different seasons of the year,

based on a large population-based registration of women with breast cancer in Denmark including detailed information on prognostic factors.

Materials and Methods

The Danish Breast Cancer Cooperative Group (DBCG) founded in 1977 is a population-based registry, which collects data on almost all cases of invasive breast cancer among residents in Denmark (a population of 5½ million, emigration and immigration rates < 2%) (www.dst.dk). Virtually all involved Danish hospital departments have applied DBCG's guidelines for diagnostic procedures, surgery, radiotherapy, adjuvant systemic therapy, and follow-up for early breast cancer. Diagnostic, therapeutic, and follow-up data have been accumulated prospectively in the DBCG registry by the use of standardized forms. The DBCG Data Center applied the same procedures for all patients, including monitoring and analysis of data, whether or not the patients participated in randomized trials [27].

Cases

The present analysis includes all women, who had a completely resected unilateral invasive carcinoma of the breast and no signs of distant metastasis as determined by routine examinations (physical examination, clinical chemistry, chest radiography, and other examinations if indicated). Cases with bilateral breast cancer were included (n=1006) and the tumour characteristics of the side with the least favourable prognostic impact were recorded in the DBCG registry. A negative sentinel node biopsy or axillary clearance (level I and II) in combination with breast-conserving surgery or mastectomy was required. Radiotherapy to the breast was

mandatory following lumpectomy. Further description of the database and treatment guidelines has been given elsewhere [28].

From June 1 1978 to May 31 2010 n=88,992 cases were registered. Of these 2176 had a diagnosis of previous breast cancer, other malignancy (except non-melanoma skin tumours) or distant metastasis and 705 patients were not operated. Further excluded from the analyses were patients with unknown tumour size (n=1373) and/or unknown axillary lymph node status (n=4775). In total n=80,149 cases were included for further analyses (Figure 1).

Variables

The seasons of surgery, generally one to three weeks after the diagnosis, was defined as follows: Winter (December 1 – February 28 or 29), spring (March 1 – May 31), summer (June 1 – August 31), and autumn (September 1 – November 30), so the summer period includes the months with the possibility of most sun exposure, due to the altitude of the sun and vacations. Treatment periods were categorized according to the national programmes initiated in: 1977, 1982, 1989, 1999, 2001, 2004, and 2007 [27]. The age at surgery was categorized in intervals: ≤ 39, 40-49, 50-59, 60-69, 70-79, and ≥ 80 years. Tumour size was categorized according to the largest tumour diameter: 0-10 mm, 11-20 mm, 21-50 mm, and ≥51 mm. The spread of breast cancer to loco-regional lymph nodes was categorized as negative, 1-3 positive lymph nodes and ≥4 positive lymph nodes. The hormone receptor status was categorized as: negative, ER or PgR positive, and unknown .

Endpoint

OS was measured from the date of surgery to the date of death. Observations were censored at emigration or at June 1, 2010, which was the date of data withdrawal of patient vital status from the Danish Centralised Civil Register.

Statistical analysis

The association between OS and season of surgery was analysed by Cox proportional hazards regression models [29;30]. The effects of season of surgery was analysed in models with an increasing level of adjustment for prognostic variables: models stratified by treatment programme (adjusted I), models stratified by treatment programme and age at surgery (adjusted II), and models stratified by treatment programme, age at surgery, hormone receptor status, and lymph node status, and further including the effects of tumour size and histological type (fully adjusted). The interpretations of a seasonal effect on survival in these models differ according to the level of adjustment. In the fully adjusted model, the seasonal effect includes the effects of unknown or not included prognostic variables including the alleged effect of vitamin D. In the adjusted II model, the seasonal effect includes the effects of both known and unknown prognostic variables. In the adjusted I model, the seasonal effect further includes the effects of referral-pattern i.e. patient age at surgery. The stratification of the Cox models was chosen to meet the proportional hazards assumption as assessed by Schoenfeld residuals plots [31]. The analyses were done for four survival periods: 0-1 years, 0-2 years, 0-5 years, and 0-10 years after surgery. The null hypothesis of no survival effect of season of surgery was assessed by the Wald chi-square statistic and a two sided p-value <0.05 was

considered statistically significant. The hazard ratios of season of surgery (winter as reference level) together with their 95% confidence intervals are reported. Analysis was performed with SAS version 9.1 (SAS Institute, Cary, NC).

Results

The person years of observation were: 77.145 for the survival period 0-1 years, 146.934 for the survival period 0-2 years, 314.754 for the survival period 0-5 years, and 493.080 for the survival period 0-10 years after surgery. For the latter group the median observation period for patients without an event was 9.3 year. The basic characteristics of the patient material according to season of surgery are presented in Table 1.

Hazard ratios of overall survival up to ten years with surgery performed in winter as reference are given in Table 2. Overall no statistically significant association between overall survival and season of surgery are observed in two, five and ten year follow-up periods. Only for the one year follow-up a significant association is observed (P = 0.029, fully adjusted analysis), overall survival is lowest for patients undergoing surgery in autumn (HR: 0.96, 95%CI: 0.86-1.08) and highest for patients undergoing surgery in summer (HR: 1.12, 95%CI: 1.00-1.26). .

Discussion

In the present study we found no evidence of a seasonal variation in the OS among more than 80.000 Danish women with primary breast cancer. The strengths of this study are the sample size, the population-based approach in a limited geographic area [32], the prospectively collected characteristics of tumor and lymph node status

and the long follow-up (mean: 9.3 years). The detailed information's offer the possibility of including season of surgery in a multivariable analysis with the variables year, age, tumor size, hormone receptor status and nodal status. Using this approach the independent prognostic effect of season of surgery seems to disappear. The limitations of the study are the lack of information about Vitamin D in the individual patient at the time of surgery. Using the estimated UV dose as surrogate for vitamin D levels must cause reservation, as it is not known, whether vitamin D levels of the breast cancer patients follow that of the background population. Lack of seasonal variation in this study does not necessarily mean, that vitamin D is not important for the OS for breast cancer patients. The vitamin D level in Danish women treated for breast cancer could be so low even among patients treated in the summer-autumn so that no difference could be detected. One nested case-control study (N = 142) showed lower vitamin D level among Danish patients at the diagnostic mammography [33]. Cross sectional studies of the plasma vitamin D in healthy Danish volunteers demonstrate a higher level in summer-autumn than in winter-spring [34]. A stronger fluctuation is found among females than among males, thus the plasma vitamin D level of more females are beneath a certain concentration and for a longer time [35].

Results from UK and Norway indicate a better prognosis if diagnosis of breast cancer takes place during the summer or autumn [36-38]. This seasonal variation was interpreted as a result of vitamin D deficiency in the dark months of the year although one author considered the possibility that the seasonal effect might be due to a relative higher rate of diagnoses in summer and the prevalence of infections

during wintertime leading to early death [39]. In contrast, results from Sweden demonstrates a worse OS for patients diagnosed in the summer probably due to a relative reduction in the number of early stage diagnoses from mammography screening which are closed in the summer month, and the health care system treating primarily the most sick patients in holiday periods[40;41].Breast cancer is regarded as a relatively slow growing cancer, with a long preclinical course [42]. If the level of vitamin D at the time of surgery should influence prognosis, the mechanism must be differences in peri-operative resistance to cancer dissemination and the logical precaution would be to ensure a high preoperative vitamin D level. However, limited evidence including the present study support this statement.

Contributors: DT contributed to conception and interpretaion of data, reviewed the literature, drafted the article and finally approved the submitted paper. KDB analysed and interpretated the data, drafted the statistical part and finally approved the submitted paper. AMT and NK contributed to the interpretation of data, revised it critically for important intellectual contents and finally approved the submitted paper.
Data sharing: The present study is based on the main part of the entire Danish Breast Cancer Cooperative Group database that has collected very complete clinical/histological and follow up data on about 85.000 Danish women with breast cancer since 1978.
We will be pleased to share the necessary data for the statistical review of our paper. However, it is not possible for us to make the entire data material public available.
We hope that your statistical reviewer will contact Karsten Bjerre: kb@dbcg.dk in order to get the basic data needed for the further review process.
Competing interest: All authors have completed the ICMJE disclosure form (available on request from the corresponding author) and declare: no support from any organisation for the submitted work; no financial relationships with any organisations that migth have an interest in the submitted work in the previous three years; no other relationships or activities that could appear to have influenced the submitted work.
Funding: This research received no specific grant from any funding agency in the public, commercial or not-for-profit sectors.

Reference List

(1) Kricker A, Armstrong B. Does sunlight have a beneficial influence on certain cancers? Prog Biophys Mol Biol 2006; 92(1):132-139.

(2) van der Rhee HJ, de Vries E, Coebergh JW. Does sunlight prevent cancer? A systematic review. Eur J Cancer 2006; 42(14):2222-2232.

(3) Thorne J, Campbell MJ. The vitamin D receptor in cancer. Proc Nutr Soc 2008; 67(2):115-127.

(4) Welsh J. Vitamin D and prevention of breast cancer. Acta Pharmacol Sin 2007; 28(9):1373-1382.

(5) Abbas S, Linseisen J, Slanger T et al. Serum 25-hydroxyvitamin D and risk of post-menopausal breast cancer--results of a large case-control study. Carcinogenesis 2008; 29(1):93-99.

(6) Bertone-Johnson ER. Vitamin D and breast cancer. Ann Epidemiol 2009; 19(7):462-467.

(7) Chen P, Hu P, Xie D et al. Meta-analysis of vitamin D, calcium and the prevention of breast cancer. Breast Cancer Res Treat 2010; 121(2):469-477.

(8) Crew KD, Gammon MD, Steck SEet al. Association between plasma 25-hydroxyvitamin D and breast cancer risk. Cancer Prev Res (Phila) 2009; 2(6):598-604.

(9) Garland CF, Garland FC, Gorham ED et al. The role of vitamin D in cancer prevention. Am J Public Health 2006; 96(2):252-261.

(10) Goodwin PJ, Ennis M, Pritchard KI et al. Prognostic effects of 25-hydroxyvitamin D levels in early breast cancer. J Clin Oncol 2009; 27(23):3757-3763.

(11) Palmieri C, MacGregor T, Girgis S et al. D. Serum 25-hydroxyvitamin D levels in early and advanced breast cancer. J Clin Pathol 2006; 59(12):1334-1336.

(12) Perez-Lopez FR, Chedraui P, Haya J. Review article: vitamin D acquisition and breast cancer risk. Reprod Sci 2009; 16(1):7-19.

(13) Rejnmark L, Tietze A, Vestergaard P et al. Reduced prediagnostic 25-hydroxyvitamin D levels in women with breast cancer: a nested case-control study. Cancer Epidemiol Biomarkers Prev 2009; 18(10):2655-2660.

(14) Chlebowski RT, Johnson KC, Kooperberg C et al. Calcium plus vitamin D supplementation and the risk of breast cancer. J Natl Cancer Inst 2008; 100(22):1581-1591.

(15) Freedman DM, Chang SC, Falk RT et al. Serum levels of vitamin D metabolites and breast cancer risk in the prostate, lung, colorectal, and ovarian cancer screening trial. Cancer Epidemiol Biomarkers Prev 2008; 17(4):889-894.

(16) Gandini S, Boniol M, Haukka J et al. Meta-analysis of observational studies of serum 25-hydroxyvitamin D levels and colorectal, breast and prostate cancer and colorectal adenoma. Int J Cancer 2011; 128(6):1414-1424.

(17) Yin L, Grandi N, Raum E et al. Meta-analysis: serum vitamin D and breast cancer risk. Eur J Cancer 2010; 46(12):2196-2205.

(18) Holmberg L, Adolfsson J, Mucci L et al. Season of diagnosis and prognosis in breast and prostate cancer. Cancer Causes Control 2009; 20(5):663-670.

(19) Lim HS, Roychoudhuri R, Peto J et al. Cancer survival is dependent on season of diagnosis and sunlight exposure. Int J Cancer 2006; 119(7):1530-1536.

(20) Porojnicu AC, Lagunova Z, Robsahm TE et al. Changes in risk of death from breast cancer with season and latitude: sun exposure and breast cancer survival in Norway. Breast Cancer Res Treat 2007; 102(3):323-328.

(21) Robsahm TE, Tretli S, Dahlback A et al. Vitamin D3 from sunlight may improve the prognosis of breast-, colon- and prostate cancer (Norway). Cancer Causes Control 2004; 15(2):149-158.

(22) Mosekilde L, Nielsen LR, Larsen ER et al. [Vitamin D deficiency. Definition and prevalence in Denmark]
Ugeskr Laeger 2005; 167(1):29-33.

(23) Moller S, Jensen MB, Ejlertsen B et al. The clinical database and the treatment guidelines of the Danish Breast Cancer Cooperative Group (DBCG); its 30-years experience and future promise. Acta Oncol 2008; 47(4):506-524.

(24) Ejlertsen B, Jensen MB, Rank F et al. Population-based study of peritumoral lymphovascular invasion and outcome among patients with operable breast cancer. J Natl Cancer Inst 2009; 101(10):729-735.

(25) Schoenfeld D. Partial Residuals for the Proportional Hazards Regression Model. Biometrika 69, 239-241. 2011.
Ref Type: Generic

(26) Kalbfleisch JD, Prentice RL. The Statistical Analysis of Failure Time Data. John Wiley.
Ref Type: Generic

(27) Kimlin MG. Geographic location and vitamin D synthesis. Mol Aspects Med 2008; 29(6):453-461.

(28) Bolland MJ, Grey AB, Ames RW et al. The effects of seasonal variation of 25-hydroxyvitamin D and fat mass on a diagnosis of vitamin D sufficiency. Am J Clin Nutr 2007; 86(4):959-964.

(29) Lambe M, Blomqvist P, Bellocco R. Seasonal variation in the diagnosis of cancer: a study based on national cancer registration in Sweden. Br J Cancer 2003; 88(9):1358-1360.

(30) Gullino PM. Natural history of breast cancer. Progression from hyperplasia to neoplasia as predicted by angiogenesis. Cancer 1977; 39(6 Suppl):2697-2703.

Table 1. Prognostic factors by season among 80,149 Danish women operated for early breast cancer 1978-2010.

	Winter		Spring		Summer		Autumn		Total	
	n	(%)	n	(%)	n	(%)	n	(%)	n	(%)
Total	18866		20120		20218		20945		80149	
Age at surgery[a]										
≤39 year	1054	(5.6)	1059	(5.3)	1004	(5.0)	1108	(5.3)	4225	(5.3)
40-49 year	3282	(17.4)	3619	(18.0)	3552	(17.6)	3655	(17.5)	14108	(17.6)
50-59 year	4926	(26.1)	5258	(26.1)	5283	(26.1)	5484	(26.2)	20951	(26.1)
60-69 year	5248	(27.8)	5524	(27.5)	5615	(27.8)	5757	(27.5)	22144	(27.6)
70-79 year	3251	(17.2)	3452	(17.2)	3557	(17.6)	3686	(17.6)	13946	(17.4)
≥80 year	1105	(5.9)	1208	(6.0)	1207	(6.0)	1255	(6.0)	4775	(6.0)
Tumor size[b]										
0-10 mm	2824	(15.0)	3108	(15.4)	2984	(14.8)	3213	(15.3)	12129	(15.1)
11-20 mm	7462	(39.6)	7999	(39.8)	8005	(39.6)	8362	(39.9)	31828	(39.7)
21-50 mm	7515	(39.8)	8006	(39.8)	8148	(40.3)	8270	(39.5)	31939	(39.8)
>50 mm	1065	(5.6)	1007	(5.0)	1081	(5.3)	1100	(5.3)	4253	(5.3)
Nodal status[c]										
Negative	9792	(51.9)	10663	(53.0)	10762	(53.2)	11250	(53.7)	42467	(53.0)
1-3 postive	5795	(30.7)	5998	(29.8)	5963	(29.5)	6055	(28.9)	23811	(29.7)
≥4 positive	3279	(17.4)	3459	(17.2)	3493	(17.3)	3640	(17.4)	13871	(17.3)
Histological gruop[d]										
Ductal grade I/?	5216	(27.6)	5498	(27.3)	5652	(28.0)	5819	(27.8)	22185	(27.7)
Ductal grade II	6911	(36.6)	7333	(36.4)	7239	(35.8)	7547	(36.0)	29030	(36.2)
Ductal grade III	3395	(18.0)	3521	(17.5)	3570	(17.7)	3667	(17.5)	14153	(17.7)
Lobular	1976	(10.5)	2141	(10.6)	2105	(10.4)	2160	(10.3)	8382	(10.5)
Other Invasive	1368	(7.3)	1627	(8.1)	1652	(8.2)	1752	(8.4)	6399	(8.0)
ER-PgR status										
Negative	2952	(15.6)	3203	(15.9)	3358	(16.6)	3264	(15.6)	12777	(15.9)
Positive	12533	(66.4)	13039	(64.8)	13125	(64.9)	13952	(66.6)	52649	(65.7)
Unknown	3381	(17.9)	3878	(19.3)	3735	(18.5)	3729	(17.8)	14723	(18.4)
Percent Er-PgR Positive[ef]		(80.9)		(80.3)		(79.6)		(81.0)		(80.5)

[a]: chisq=12.7, df=15, P=0.62.

[b]:chisq=14.0, df=9, P=0.12.

[c]:chisq=18.1, df=6, P=0.006.

[d]:chisq=24.4, df=12, P=0.019.

[e]: Positive relative to sum of positive and negative.

[f]: chisq=13.5, df=3, P=0.004.

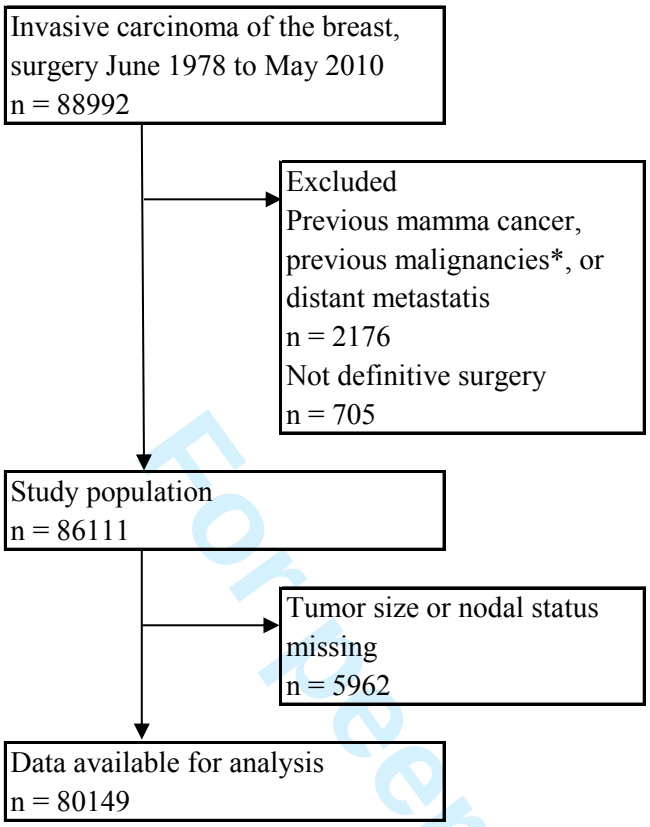
Table 2. Overall survival (hazard ratio) by Cox proportional hazards regression at survival periods 0-1, 0-2, 0-5, and 0-10 years post surgery. Estimates of season of surgery are shown among 80,149 Danish women operated for breast cancer 1978-2010.

Period of follow up	Adjusted I[a]			Adjusted II[b]			Fully adjusted[c]		
Season of surgery	HR	95% CI	P	HR	95% CI	P	HR	95% CI	P
0-1 years post surgery									
Winter	1	(reference)	0.039	1	(reference)	0.046	1	(reference)	0.029
Spring	1.06	(0.94- 1.18)		1.05	(0.93- 1.17)		1.07	(0.95- 1.20)	
Summer	1.09	(0.98- 1.22)		1.08	(0.97- 1.21)		1.12	(1.00- 1.26)	
Autumn	0.94	(0.84- 1.05)		0.93	(0.83- 1.04)		0.96	(0.86- 1.08)	
0-2 years post surgery									
Winter	1	(reference)	0.097	1	(reference)	0.075	1	(reference)	0.24
Spring	0.97	(0.90- 1.04)		0.97	(0.90- 1.04)		0.99	(0.92- 1.06)	
Summer	0.99	(0.92- 1.06)		0.98	(0.91- 1.05)		1.00	(0.93- 1.08)	
Autumn	0.92	(0.86- 0.99)		0.91	(0.85- 0.98)		0.94	(0.88- 1.01)	
0-5 years post surgery									
Winter	1	(reference)	0.72	1	(reference)	0.57	1	(reference)	0.89
Spring	0.99	(0.95- 1.03)		0.99	(0.95- 1.03)		1.00	(0.96- 1.05)	
Summer	0.99	(0.95- 1.03)		0.99	(0.94- 1.03)		1.01	(0.96- 1.05)	
Autumn	0.98	(0.93- 1.02)		0.97	(0.93- 1.01)		0.99	(0.95- 1.03)	
0-10 years post surgery									
Winter	1	(reference)	0.84	1	(reference)	0.73	1	(reference)	0.79
Spring	1.00	(0.96- 1.03)		1.00	(0.96- 1.03)		1.01	(0.98- 1.05)	
Summer	1.00	(0.97- 1.03)		1.00	(0.96- 1.03)		1.01	(0.98- 1.05)	
Autumn	0.99	(0.95- 1.02)		0.98	(0.95- 1.02)		1.00	(0.97- 1.04)	

[a]: Model stratified for treatment programme.

[b]: Model stratified for treatment programme and age at surgery.

[c]: Model stratified for treatment programme, age at surgery, hormone receptor status, and nodal status and including the effects of tumor size and histological group.



* Except non-melanoma skin tumours

Figure 1. Flow-diagram: prospective registretion of Danish women operated for early breast cancer 1778-2010

STROBE 2007 (v4) Statement—Checklist of items that should be included in reports of *cohort study* BMJopen-2011-000358

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	1
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	2
Objectives	3	State specific objectives, including any prespecified hypotheses	2-3
Methods			
Study design	4	Present key elements of study design early in the paper	3
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	3
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up	3-4
		(*b*) For matched studies, give matching criteria and number of exposed and unexposed	
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	3-4
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	4
Bias	9	Describe any efforts to address potential sources of bias	
Study size	10	Explain how the study size was arrived at	4-5
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	4
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	5
		(*b*) Describe any methods used to examine subgroups and interactions	5
		(*c*) Explain how missing data were addressed	
		(*d*) If applicable, explain how loss to follow-up was addressed	
		(*e*) Describe any sensitivity analyses	5-6
Results			

Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	6 + fig. 1
		(b) Give reasons for non-participation at each stage	fig.1
		(c) Consider use of a flow diagram	fig.1
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	Table 1
		(b) Indicate number of participants with missing data for each variable of interest	4 + fig. 1
		(c) Summarise follow-up time (eg, average and total amount)	6
Outcome data	15*	Report numbers of outcome events or summary measures over time	Table 2
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	6 + table 2
		(*b*) Report category boundaries when continuous variables were categorized	4 + table 2
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	
Discussion			
Key results	18	Summarise key results with reference to study objectives	6-7
Limitations			
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	7
Generalisability	21	Discuss the generalisability (external validity) of the study results	7
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	No funding

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

Breast cancer survival and season of surgery: an ecological, open cohort study

Journal:	*BMJ Open*
Manuscript ID:	bmjopen-2011-000358.R1
Article Type:	Research
Date Submitted by the Author:	14-Nov-2011
Complete List of Authors:	Teilum, Dorthe; Brystkirurgisk Klinik PBB , Bjerre, Karsten; Danish Breast Cancer Cooperative Group, Department 2501, Tjønneland, Anne; Institute of Cancer Epidemiology,, Dannish Cancer Society Kroman, Niels; Rigshospitalet,,
Primary Subject Heading:	Surgery
Secondary Subject Heading:	Oncology
Keywords:	Breast cancer, Breast surgery < SURGERY, Vitamin D

Breast cancer survival and season of surgery: an ecological, open cohort study

Dorthe Teilum, Karsten D Bjerre, Anne M Tjønneland, and Niels Kroman

Abstract

Background: Vitamin D has been suggested to influence the incidence and prognosis of breast cancer and studies have found better overall survival after diagnosis for breast cancer in summer-autumn, where the vitamin D level are expected to be highest.

Objective: To compare the prognostic outcome for early breast cancer patients operated at different seasons of the year.

Design: Open population-based cohort study.

Setting: Danish women operated 1978-2010.

Cases: **79,658** adjusted for age at surgery, period of surgery, tumor size, axillary lymph node status, and hormone receptor status.

Statistical analysis: The association between overall survival and season of surgery was analyzed by Cox proportional hazards regression models, at survival periods 0-1, 0-2, 0-5 and 0-10 years after surgery. A two sided p-value < 0.05 was considered statistical significant.

Results: ***Only after adjustment for prognostic factors that may be influenced by vit D one year survival was close to significantly associated with season of surgery*. Two, five and ten years after surgery the association between overall survival and season of surgery was not significant.**

Limitations: Season is a surrogate measure of vitamin D.

Conclusion: We found no evidence of a seasonal variation in the survival after surgery for early breast cancer. Lack of seasonal variation in this study does not necessarily mean, that vitamin D is of no importance for the outcome for breast cancer patients.

Article summary

Article focus:

- Breast cancer survival and season of surgery

Key message:

- No evidence of a seasonal variation

Strengths of this study:

- The sample size (**approximately** 80.000 cases)
- The population-based approach in a limited geographic area
- The prospectively collected characteristics of tumor and lymph node status
- The long follow-up (**median 10.0** years)

Limitations of this study:

- The lack of information about vitamin D **status** in the individual patient at the time of surgery
- It is not known, whether vitamin D levels of the breast cancer patients follow that of the background population

Introduction

Over the past decades ecological studies have inspired to the hypothesis that exposure to sunlight and hence difference in **serum** vitamin D, may influence both risk and prognosis for breast cancer [1;2]. The hypothesis has been supported by several in vitro and animal studies [3;4], in addition to case-control and cohort studies with measurements of **vitamin D as serum** 25 hydroxy-vitamin D (25(OH)D)[5-15] although not all studies including two meta-analyses could support these findings[16-19]. **Four studies found** the prognosis of breast cancer to vary with the season for diagnosis. **The** three **of them** found that patients diagnosed in summer-autumn had a better disease outcome than patients diagnosed in winter-spring [20-22] and one study found a higher overall mortality for patients diagnosed in **late summer** compared to those diagnosed in **mid winter**[23].

In Denmark, positioned at 55-58^0 northern latitude, there is not sufficient sun to **synthesise** vitamin D in the human skin during 6-8 months of the year. Measurements of vitamin D in healthy Danish volunteers demonstrate a pronounced seasonal variation of vitamin D with a maximum in **late summer** and a minimum in **early spring**, which indicates, that the content of vitamin D in the average Danish diet could not compensate for the lack of sun induced vitamin D production during wintertime [24].

If the vitamin D **status** at the time of the operation is important for the overall survival (OS), it should be both easy and inexpensive to adjust preoperatively. The aim of this study is to compare the prognostic outcome for early breast cancer patients diagnosed and operated at different seasons of the year based on

a large population-based registration of women with breast cancer in Denmark including detailed information on prognostic factors.

Materials and Methods

The Danish Breast Cancer Cooperative Group (DBCG) founded in 1977 is a population-based registry, which collects data on almost all cases of invasive breast cancer among residents in Denmark (a population of 5½ million, emigration and immigration rates < 2%) (www.dst.dk). Virtually all involved Danish hospital departments have applied DBCG's guidelines for diagnostic procedures, surgery, radiotherapy, adjuvant systemic therapy, and follow-up for early breast cancer. Diagnostic, therapeutic, and follow-up data have been accumulated prospectively in the DBCG registry by the use of standardized forms. The DBCG Data Center applied the same procedures for all patients, including monitoring and analysis of data, whether or not the patients participated in randomized trials [25].

Cases

The present analysis includes all women, who had a completely resected invasive carcinoma of the breast and no signs of distant metastasis as determined by routine examinations (physical examination, clinical chemistry, chest radiography, and other examinations if indicated). Cases with bilateral breast cancer were included (n=**1535**) and the tumour characteristics of the side with the least favourable prognostic impact were recorded in the DBCG registry. A negative sentinel node biopsy or axillary clearance (level I and II) in combination with breast-conserving surgery or mastectomy was required. Radiotherapy to the

breast was mandatory following lumpectomy. Further description of the database and treatment guidelines has been given elsewhere [25;26].

From June 1 1978 to May 31 2010 n=**89,409** cases were registered. Of these **3113** had a diagnosis of previous breast cancer, other malignancy (except non-melanoma skin tumours) or distant metastasis and **610** patients were not operated. Further excluded from the analyses were patients with unknown tumour size (n=**2045**) and/or unknown axillary lymph node status (n=**5678**). In total n=**79,658** cases were included for further analyses (Figure 1).

Variables

The seasons of surgery, generally one to three weeks after the diagnosis, was defined as follows: Winter (December 1 – February 28 or 29), spring (March 1 – May 31), summer (June 1 – August 31), and autumn (September 1 – November 30), so the summer period includes the months with the possibility of most sun exposure, due to the altitude of the sun and vacations. Treatment periods were categorized according to the national programmes initiated in: 1977, 1982, 1989, 1999, 2001, 2004, and 2007 [25]. The age at surgery was categorized in intervals: ≤ 39, 40-49, 50-59, 60-69, 70-79, and ≥ 80 years. Tumour size was categorized according to the largest tumour diameter: 0-10 mm, 11-20 mm, 21-50 mm, and ≥51 mm. The spread of breast cancer to loco-regional lymph nodes was categorized as negative, 1-3 positive lymph nodes and ≥4 positive lymph nodes. The hormone receptor status was categorized as: negative, ER or PgR positive, and unknown . **The histopathological status was categorized in five groups as: grade I, II, or III ductal carcinoma, lobular carcinoma, and carcinoma of**

other types or unknown diagnosis. The frequency of allocated systemic treatment (chemotherapy and endocrine therapy) by season of surgery was reported.

Endpoint

OS was measured from the date of surgery to the date of death. Observations were censored at emigration or at June 1, **2011**, which was the date of data withdrawal of patient vital status from the Danish Centralised Civil Register.

Statistical analysis

The association between OS and season of surgery was analysed by Cox proportional hazards regression models [27;28]. The effects of season of surgery was analysed in models with an increasing level of adjustment for prognostic variables: models stratified by treatment programme (adjusted I), models stratified by treatment programme and age at surgery (adjusted II), and models stratified by treatment programme, age at surgery, hormone receptor status, and lymph node status, and further including the effects of tumour size and histological type (fully adjusted). The interpretations of a seasonal effect on survival in these models differ according to the level of adjustment. In the fully adjusted model, the seasonal effect includes the effects of unknown or not included prognostic variables including the alleged effect of vitamin D. In the adjusted II model, the seasonal effect includes the effects of both known and unknown prognostic variables. In the adjusted I model, the seasonal effect further includes the effects of referral-pattern i.e. patient age at surgery. The stratification of the Cox models

was chosen to meet the proportional hazards assumption as assessed by Schoenfeld residuals plots[27]. The analyses were done for four survival periods: 0-1 years, 0-2 years, 0-5 years, and 0-10 years after surgery. The null hypothesis of no survival effect of season of surgery was assessed by the Wald chi-square statistic and a two sided p-value <0.05 was considered statistically significant. The hazard ratios of season of surgery (winter as reference level) together with their 95% confidence intervals are reported. **Due to the long period of inclusion the potential heterogeneity of seasonal effects according to period of inclusion was investigated in models including an interaction term of season of surgery and programme series (1977 and 1982 versus 1989 versus 1999, 2001, 2004 and 2007).** Analysis was performed with SAS version 9.1 (SAS Institute, Cary, NC).

Results

The person years of observation were: **78,587** for the survival period 0-1 years, **151,980** for the survival period 0-2 years, **327,646** for the survival period 0-5 years, and **516,011** for the survival period 0-10 years after surgery. For the latter group the median observation period for patients without an event was **10.0** year. The basic characteristics of the patient material according to season of surgery are presented in Table 1.

Hazard ratios of overall survival up to ten years with surgery performed in winter as reference are given in Table 2. Overall no statistically significant association between overall survival and season of surgery are observed in two, five and ten year follow-up periods. Only for the one year follow-up a **close to** significant

association is observed (*P* = **0.052**, fully adjusted analysis), overall survival is **highest** for patients undergoing surgery in autumn (HR: **0.97**, 95%CI: 0.86-**1.09**) and **lowest** for patients undergoing surgery in summer (HR: 1.12, 95%CI: 1.00-**1.26**). **Heterogeneity of seasonal effects according to period of inclusion was not statistical significant irrespective of model adjustment or survival period.**

Discussion

In the present study we found no evidence of a seasonal variation in the OS among **almost** 80.000 Danish women with primary breast cancer. The strengths of this study are the sample size, the population-based approach in a limited geographic area [29], the prospectively collected characteristics of tumor and lymph node status and the long follow-up (**median 10.0** years). The detailed information's offer the possibility of including season of surgery in a multivaria**te** analysis with the variables year, age **at surgery**, tumor size, **nodal status**, hormone receptor status and **histopathological type**. **It should be noted that in our analysis, the "Adjusted II" models are stratified by treatment programme and age at surgery only. Thus, the estimates of association between OS and seasonal of surgery are not affected by the variables potentially associated with D-vitamin or season of surgery (tumor size, positive axillary nodes, high grade tumors and ER-/Pr- status).** Using this approach the independent prognostic effect of season of surgery seems to disappear. The limitations of the study are the lack of information about **serum** Vitamin D in the individual patient at the time of surgery. Using the estimated UV dose as surrogate for vitamin D **status** must cause reservation, as it is not known,

whether vitamin D **status** of the breast cancer patients follow that of the background population. Lack of seasonal variation in this study does not necessarily mean, that vitamin D is not important for the OS for breast cancer patients. The **serum** vitamin D in Danish women treated for breast cancer could be so low even among patients treated in the summer-autumn so that no difference could be detected. One nested case-control study (N = 142) showed lower **serum** vitamin D among Danish patients at the diagnostic mammography [14]. Cross sectional studies of the plasma vitamin D in healthy Danish volunteers demonstrate a higher level in summer-autumn than in winter-spring [24]. Results from UK and Norway indicate a better prognosis if diagnosis of breast cancer takes place during the summer or autumn [20-22]. This seasonal variation was interpreted as a result of vitamin D deficiency in the dark months of the year although one author considered the possibility that the seasonal effect might be due to a relative higher rate of diagnoses in summer and the prevalence of infections during wintertime leading to early death [20]. In contrast, results from Sweden demonstrates a worse OS for patients diagnosed in the summer probably due to a relative reduction in the number of early stage diagnoses from mammography screening which are closed in the summer month**s**, and the health care system treating primarily the most sick patients in holiday periods[23;30]. Breast cancer is regarded as a relatively slow growing cancer, with a long preclinical course [31]. **If vitamin D level should be of etiologic or prognostic importance it is supposed that the influence is working over a longer time period and not just reflected by vitamin D status at time of diagnosis.** If the level of vitamin D at the time of surgery should influence prognosis, the mechanism must be

differences in peri-operative resistance to cancer dissemination and the logical precaution would be to ensure a high preoperative vitamin D level. However, limited evidence including the present study support this statement.

Contributors: DT contributed to conception and interpretation of data, reviewed the literature, drafted the article and finally approved the submitted paper. KDB analysed and interpretated the data, drafted the statistical part and finally approved the submitted paper. AMT and NK contributed to the interpretation of data, revised it critically for important intellectual contents and finally approved the submitted paper.
Data sharing: **No additional data available.**
We will be pleased to share the necessary data for the statistical review of our paper. However, it is not possible for us to make the entire data material public available.
Competing interest: All authors have completed the ICMJE disclosure form (available on request from the corresponding author) and declare: no support from any organisation for the submitted work; no financial relationships with any organisations that might have an interest in the submitted work in the previous three years; no other relationships or activities that could appear to have influenced the submitted work.
Funding: This research received no specific grant from any funding agency in the public, commercial or not-for-profit sectors.

Reference List

(1) Kricker A, Armstrong B. Does sunlight have a beneficial influence on certain cancers? Prog Biophys Mol Biol 2006; 92(1):132-139.

(2) van der Rhee HJ, de VE, Coebergh JW. Does sunlight prevent cancer? A systematic review. Eur J Cancer 2006; 42(14):2222-2232.

(3) Thorne J, Campbell MJ. The vitamin D receptor in cancer. Proc Nutr Soc 2008; 67(2):115-127.

(4) Welsh J. Vitamin D and prevention of breast cancer. Acta Pharmacol Sin 2007; 28(9):1373-1382.

(5) Abbas S, Linseisen J, Slanger T, Kropp S, Mutschelknauss EJ, Flesch-Janys D et al. Serum 25-hydroxyvitamin D and risk of post-menopausal breast cancer--results of a large case-control study. Carcinogenesis 2008; 29(1):93-99.

(6) Bertone-Johnson ER. Vitamin D and breast cancer. Ann Epidemiol 2009; 19(7):462-467.

(7) Chen P, Hu P, Xie D, Qin Y, Wang F, Wang H. Meta-analysis of vitamin D, calcium and the prevention of breast cancer. Breast Cancer Res Treat 2010; 121(2):469-477.

(8) Crew KD, Gammon MD, Steck SE, Hershman DL, Cremers S, Dworakowski E et al. Association between plasma 25-hydroxyvitamin D and breast cancer risk. Cancer Prev Res (Phila Pa) 2009; 2(6):598-604.

(9) Garland CF, Garland FC, Gorham ED, Lipkin M, Newmark H, Mohr SB et al. The role of vitamin D in cancer prevention. Am J Public Health 2006; 96(2):252-261.

(10) Goodwin PJ, Ennis M, Pritchard KI, Koo J, Hood N. Prognostic effects of 25-hydroxyvitamin D levels in early breast cancer. J Clin Oncol 2009; 27(23):3757-3763.

(11) Kim HJ, Lee YM, Ko BS, Lee JW, Yu JH, Son BH et al. Vitamin D deficiency is correlated with poor outcomes in patients with luminal-type breast cancer. Ann Surg Oncol 2011; 18(7):1830-1836.

(12) Palmieri C, MacGregor T, Girgis S, Vigushin D. Serum 25-hydroxyvitamin D levels in early and advanced breast cancer. J Clin Pathol 2006; 59(12):1334-1336.

(13) Perez-Lopez FR, Chedraui P, Haya J. Review article: vitamin D acquisition and breast cancer risk. Reprod Sci 2009; 16(1):7-19.

(14) Rejnmark L, Tietze A, Vestergaard P, Buhl L, Lehbrink M, Heickendorff L et al. Reduced prediagnostic 25-hydroxyvitamin D levels in women with breast cancer: a nested case-control study. Cancer Epidemiol Biomarkers Prev 2009; 18(10):2655-2660.

(15) Vrieling A, Hein R, Abbas S, Schneeweiss A, Flesch-Janys D, Chang-Claude J. Serum 25-hydroxyvitamin D and postmenopausal breast cancer survival: a prospective patient cohort study. Breast Cancer Res 2011; 13(4):R74.

(16) Chlebowski RT, Johnson KC, Kooperberg C, Pettinger M, Wactawski-Wende J, Rohan T et al. Calcium plus vitamin D supplementation and the risk of breast cancer. J Natl Cancer Inst 2008; 100(22):1581-1591.

(17) Freedman DM, Chang SC, Falk RT, Purdue MP, Huang WY, McCarty CA et al. Serum levels of vitamin D metabolites and breast cancer risk in the prostate, lung, colorectal, and ovarian cancer screening trial. Cancer Epidemiol Biomarkers Prev 2008; 17(4):889-894.

(18) Gandini S, Boniol M, Haukka J, Byrnes G, Cox B, Sneyd MJ et al. Meta-analysis of observational studies of serum 25-hydroxyvitamin D levels and colorectal, breast and prostate cancer and colorectal adenoma. Int J Cancer 2011; 128(6):1414-1424.

(19) Yin L, Grandi N, Raum E, Haug U, Arndt V, Brenner H. Meta-analysis: serum vitamin D and breast cancer risk. Eur J Cancer 2010; 46(12):2196-2205.

(20) Lim HS, Roychoudhuri R, Peto J, Schwartz G, Baade P, Moller H. Cancer survival is dependent on season of diagnosis and sunlight exposure. Int J Cancer 2006; 119(7):1530-1536.

(21) Porojnicu AC, Lagunova Z, Robsahm TE, Berg JP, Dahlback A, Moan J. Changes in risk of death from breast cancer with season and latitude: sun exposure and breast cancer survival in Norway. Breast Cancer Res Treat 2007; 102(3):323-328.

(22) Robsahm TE, Tretli S, Dahlback A, Moan J. Vitamin D3 from sunlight may improve the prognosis of breast-, colon- and prostate cancer (Norway). Cancer Causes Control 2004; 15(2):149-158.

(23) Holmberg L, Adolfsson J, Mucci L, Garmo H, Adami HO, Moller H et al. Season of diagnosis and prognosis in breast and prostate cancer. Cancer Causes Control 2009; 20(5):663-670.

(24) Mosekilde L, Nielsen LR, Larsen ER, Moosgaard B, Heickendorff L. [Vitamin D deficiency. Definition and prevalence in Denmark] Ugeskr Laeger 2005; 167(1):29-33.

(25) Moller S, Jensen MB, Ejlertsen B, Bjerre KD, Larsen M, Hansen HB et al. The clinical database and the treatment guidelines of the Danish Breast Cancer Cooperative Group (DBCG); its 30-years experience and future promise. Acta Oncol 2008; 47(4):506-524.

(26) Ejlertsen B, Jensen MB, Rank F, Rasmussen BB, Christiansen P, Kroman N et al. Population-based study of peritumoral lymphovascular invasion and outcome among patients with operable breast cancer. J Natl Cancer Inst 2009; 101(10):729-735.

(27) Schoenfeld D. Partial Residuals for the Proportional Hazards Regression Model. Biometrika 69, 239-241. 2011.
Ref Type: Generic

(28) Kalbfleisch JD, Prentice RL. The Statistical Analysis of Failure Time Data. John Wiley.
Ref Type: Generic

(29) Kimlin MG. Geographic location and vitamin D synthesis. Mol Aspects Med 2008; 29(6):453-461.

(30) Lambe M, Blomqvist P, Bellocco R. Seasonal variation in the diagnosis of cancer: a study based on national cancer registration in Sweden. Br J Cancer 2003; 88(9):1358-1360.

(31) Gullino PM. Natural history of breast cancer. Progression from hyperplasia to neoplasia as predicted by angiogenesis. Cancer 1977; 39(6 Suppl):2697-2703.

Table 1. Prognostic factors by season among **79,658** Danish women operated for early breast cancer between **June 1, 1978 and May 31,** 2010

	Winter		Spring		Summer		Autumn		Total	
Characteristic	n	(%)	n	(%)	n	(%)	n	(%)	n	(%)
Total	18760		20067		20033		20798		79658	
Age at surgery[a]										
≤39 year	1051	(5.6)	1057	(5.3)	1001	(5.0)	1094	(5.3)	4203	(5.3)
40-49 year	3249	(17.3)	3604	(18.0)	3524	(17.6)	3637	(17.5)	14014	(17.6)
50-59 year	4906	(26.2)	5251	(26.2)	5232	(26.1)	5461	(26.3)	20850	(26.2)
60-69 year	5203	(27.7)	5506	(27.4)	5520	(27.6)	5702	(27.4)	21931	(27.5)
70-79 year	3233	(17.2)	3436	(17.1)	3541	(17.7)	3642	(17.5)	13852	(17.4)
≥80 year	1118	(6.0)	1213	(6.0)	1215	(6.1)	1262	(6.1)	4808	(6.0)
Period of surgery[b]										
1977-1989	4592	(24.5)	4783	(23.8)	5115	(25.5)	5448	(26.2)	19938	(25.0)
1990-1999	5626	(30.0)	6160	(30.7)	6359	(31.7)	6559	(31.5)	24704	(31.0)
2000-2010	8542	(45.5)	9124	(45.5)	8559	(42.7)	8791	(42.3)	35016	(44.0)
Tumor size[c]										
0-10 mm	2832	(15.1)	3136	(15.6)	2972	(14.8)	3211	(15.4)	12151	(15.3)
11-20 mm	7419	(39.5)	7983	(39.8)	7945	(39.7)	8310	(40.0)	31657	(39.7)
21-50 mm	7469	(39.8)	7964	(39.7)	8053	(40.2)	8201	(39.4)	31687	(39.8)
>50 mm	1040	(5.5)	984	(4.9)	1063	(5.3)	1076	(5.2)	4163	(5.2)
Nodal status[d]										
Negative	9767	(52.1)	10672	(53.2)	10723	(53.5)	11233	(54.0)	42395	(53.2)
1-3 postive	5772	(30.8)	5984	(29.8)	5915	(29.5)	6015	(28.9)	23686	(29.7)
≥4 positive	3221	(17.2)	3411	(17.0)	3395	(16.9)	3550	(17.1)	13577	(17.0)
Histological gruop[e]										
Ductal grade I	4808	(25.6)	5129	(25.6)	5242	(26.2)	5390	(25.9)	20569	(25.8)
Ductal grade II/?[f]	7268	(38.7)	7672	(38.2)	7542	(37.6)	7893	(38.0)	30375	(38.1)
Ductal grade III	3351	(17.9)	3504	(17.5)	3517	(17.6)	3626	(17.4)	13998	(17.6)
Lobular	1963	(10.5)	2135	(10.6)	2086	(10.4)	2137	(10.3)	8321	(10.4)
Other Invasive	1370	(7.3)	1627	(8.1)	1646	(8.2)	1752	(8.4)	6395	(8.0)
ER-PgR status										
Negative	2919	(15.6)	3176	(15.8)	3299	(16.5)	3217	(15.5)	12611	(15.8)
Positive	12453	(66.4)	13054	(65.1)	12994	(64.9)	13849	(66.6)	52350	(65.7)

Unknown	3388	(18.1)	3837	(19.1)	3740	(18.7)	3732	(17.9)	14697	(18.5)
Percent Er-PgR Positive[gh]		(81.0)		(80.4)		(79.8)		(81.1)		(80.6)
Adjuvant systemic thearpy										
None	9449	(50.4)	10256	(51.1)	10551	(52.7)	10940	(52.6)	41196	(51.7)
Chemotherapy[i]	4749	(25.3)	5063	(25.2)	4849	(24.2)	5043	(24.2)	19704	(24.7)
Endocrine therapy[j]	6270	(33.4)	6629	(33.0)	6347	(31.7)	6654	(32.0)	25900	(32.5)

[a] chisq=12.2, df=15, P=0.66.

[b] chisq=80.7, df=6, P=0.0001.

[c] chisq=14.9, df=9, P=0.09.

[d] chisq=19.5, df=6, P=0.003.

[e] chisq=25.1, df=12, P=0.014.

[f] Unknown grade n=1533.

[g] Positive relative to sum of positive and negative.

[h] chisq=12.7, df=3, P=0.005.

[i] chisq=11.7, df=3, P=0.009.

[h] chisq=18.4, df=3, P=0.0004.

Table 2. Overall survival by Cox proportional hazards regression at survival periods 0-1, 0-2, 0-5, and 0-10 years post surgery. Estimates of season of surgery are shown among **79,658** Danish women operated for breast cancer **between June 1,** 1978 **and May 31**, 2010.

Period of follow up	Adjusted I[a]			Adjusted II[b]			Fully adjusted[c]		
Season of surgery	HR	95% CI	P	HR	95% CI	P	HR	95% CI	P
0-1 years after surgery									
Winter	1	(reference)	0.053	1	(reference)	0.067	1	(reference)	0.052
Spring	1.07	(0.95- 1.20)		1.06	(0.95- 1.19)		1.07	(0.96- 1.20)	
Summer	1.09	(0.97- 1.22)		1.08	(0.96- 1.21)		1.12	(1.00- 1.25)	
Autumn	0.95	(0.84- 1.06)		0.94	(0.84- 1.06)		0.97	(0.86- 1.09)	
0-2 years after surgery									
Winter	1	(reference)	0.19	1	(reference)	0.17	1	(reference)	0.43
Spring	0.99	(0.92- 1.06)		0.98	(0.92- 1.06)		1.00	(0.93- 1.07)	
Summer	0.99	(0.92- 1.06)		0.99	(0.92- 1.06)		1.01	(0.94- 1.08)	
Autumn	0.93	(0.87- 1.00)		0.93	(0.86- 1.00)		0.96	(0.89- 1.03)	
0-5 years after surgery									
Winter	1	(reference)	0.60	1	(reference)	0.48	1	(reference)	0.96
Spring	0.98	(0.94- 1.03)		0.98	(0.94- 1.03)		1.00	(0.95- 1.04)	
Summer	0.98	(0.94- 1.02)		0.97	(0.93- 1.02)		1.00	(0.95- 1.04)	
Autumn	0.97	(0.93- 1.01)		0.97	(0.93- 1.01)		0.99	(0.95- 1.03)	
0-10 years after surgery									
Winter	1	(reference)	0.90	1	(reference)	0.81	1	(reference)	0.92
Spring	1.00	(0.96- 1.03)		1.00	(0.96- 1.03)		1.01	(0.98- 1.05)	
Summer	1.00	(0.96- 1.03)		0.99	(0.96- 1.03)		1.01	(0.98- 1.05)	
Autumn	0.99	(0.95- 1.02)		0.98	(0.95- 1.02)		1.00	(0.97- 1.04)	

[a] Model stratified for treatment programme.

[b] Model stratified for treatment programme and age at surgery.

[c] Model stratified for treatment programme, age at surgery, hormone receptor status, and nodal status and including the effects of tumor size and histological group.

STROBE 2007 (v4) Statement—Checklist of items that should be included in reports of *cohort study* BMJopen-2011-000358

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	1
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	2
Objectives	3	State specific objectives, including any prespecified hypotheses	2-3
Methods			
Study design	4	Present key elements of study design early in the paper	3
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	3
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up	3-4
		(*b*) For matched studies, give matching criteria and number of exposed and unexposed	
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	3-4
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	4
Bias	9	Describe any efforts to address potential sources of bias	
Study size	10	Explain how the study size was arrived at	4-5
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	4
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	5
		(*b*) Describe any methods used to examine subgroups and interactions	5
		(*c*) Explain how missing data were addressed	
		(*d*) If applicable, explain how loss to follow-up was addressed	
		(*e*) Describe any sensitivity analyses	5-6
Results			

Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	6 + fig. 1
		(b) Give reasons for non-participation at each stage	fig.1
		(c) Consider use of a flow diagram	fig.1
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	Table 1
		(b) Indicate number of participants with missing data for each variable of interest	4 + fig. 1
		(c) Summarise follow-up time (eg, average and total amount)	6
Outcome data	15*	Report numbers of outcome events or summary measures over time	Table 2
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	6 + table 2
		(*b*) Report category boundaries when continuous variables were categorized	4 + table 2
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	
Discussion			
Key results	18	Summarise key results with reference to study objectives	6-7
Limitations			
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	7
Generalisability	21	Discuss the generalisability (external validity) of the study results	7
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	No funding

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.